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Comparison of Protoscolex Hook Morphologies in Human, Sheep, and Cattle *Echinococcus granulosus* Isolates

İnsan, Koyun ve Sığır Echinococcus granulosus İzolatlarında Protoskoleks Çengel Morfolojilerinin Karşılaştırılması

Ahmed Galip Halidi[1], Hasan Yılmaz[2], Yunus Emre Beyhan[*2]*

[1]Muş Alparslan University, Bulanık Vocational School, Muş, Türkiye

[2]Department of Parasitology, Van Yüzüncü Yıl University Faculty of Medicine, Van, Türkiye

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ABSTRACT

Objective: Cystic echinococcosis (CE) is an important parasitic infection caused by *Echinococcus granulosus* (*E. granulosus*) larvae, adults of which live in Canidae. Although CE is observed in all over the world, it is more prevalent in developing and underdeveloped nations.

Material and Method: This study was conducted on a total of 60 liver cyst samples, including 20 sheep and 20 cattle slaughtered in the abattoirand 20 patients operated with the diagnosis of KE in University Medical Center between the dates June 2018 and April 2019. The collected cyst fluids were centrifuged and protoscolices were collected. Large and small hook lengths (LHL, SHL), widths (LHW, SHW), and blade lengths (LHBL, SHBL) were measured. The results were evaluated with the SPSS program.

Results: The average of human, sheep and bovine LHL measurements were 21.710 ± 1.073 µm, 24.322 ± 1.073 µm, 25.223 ± 1.073 µm; SHL measurements were 16.946 ± 0.840 µm, 20.746 ± 0.911 µm, 21.199 ± 0.977 µm; LHW measurements were 5.437 ± 0.358 µm, 7.817 ± 0.729 µm, 8.528 ± 0.589 µm, respectively; SHW measurements were 7.229 ± 0.631 µm, 6.417 ± 0.507 µm, 6.488 ± 0.463 µm, respectively; LHBL measurements were 13.236 ± 0.746 µm, 13.862 ± 0.767 µm, 13.345 ± 0.728 µm; SHBL measurements were determined as 8.918 ± 0.471 µm, 9.414 ± 0.483 µm, 9.457 ± 0.476 µm, respectively. The length, width and blade lengths of large and small hooks isolated from human, sheep and cattle were significant difference between all groups. When hook morphology measurements were grouped in pairs as human-sheep, human-cattle and sheep-cattle and analyzed statistically; it was determined that LHL, SHL, SHW and SHBL were significantly different between human-cattle and human-sheep, but not between sheep-cattle. It was found that LHW was significantly different between human-cattle and sheep-cattle, while LHBL was significantly different only between human-sheep.

Conclusion: The morphological features of the large and small hooks of *E. granulosus* may represent morphological adaptation in vertebrate hosts. For this reason, it is thought that morphological parameters can be useful in the differentiation of isolates and can be used together with molecular studies in the future.
***Keywords*:** *Echinococcus granulosus*, Hydatid cyst, Protoscolex, Hook, Morphology

ÖZET

Giriş: Kistik ekinokokkoz (KE), erişkinleri köpekgillerde yaşayan *Echinococcus granulosus* (*E. granulosus*) larvalarının neden olduğu önemli bir paraziter enfeksiyondur. Tüm dünyada görülmesine rağmen, gelişmekte olan ve az gelişmiş ülkelerde daha yaygındır.

Materyal ve Metot: Bu çalışma, Üniversite Tıp Merkezi'nde Haziran 2018-Nisan 2019 tarihleri arasında mezbahada kesilen 20 koyun ve 20 sığır ve KE tanısı ile opere edilen 20 hasta olmak üzere toplam 60 karaciğer kist örneği üzerinde yapıldı. Toplanan kist sıvıları santrifüj edildi ve protoskoleksler toplandı. Büyük ve küçük kanca uzunlukları (BKU, KKU), genişlikleri (BKG, KKG), bıçak uzunlukları (BKBU, KKBU) ölçülmüştür. Bu konakçıların her biri için 20 büyük ve 20 küçük kancanın uzunluğu, genişliği ve bıçak uzunlukları ölçüldü. Ölçüm sonuçları SPSS program ile değerlendirildi.

Bulgular: İnsan, koyun ve sığır BKU ölçümleri 21,710 ± 1,073 µm, 24,322 ± 1,073 µm, 25,223 ± 1,073 µm; KKU ölçümleri 16,946 ± 0,840 µm, 20,746 ± 0,911 µm, 21,199 ± 0,977 µm; BKG ölçümleri sırasıyla 5,437 ± 0,358 µm, 7,817 ± 0,729 µm, 8,528 ± 0,589 µm idi; KKG ölçümleri sırasıyla 7,229 ± 0,631 µm, 6,417 ± 0,507 µm, 6,488 ± 0,463 µm; BKBU ölçümleri 13,236 ± 0,746 µm, 13,862 ± 0,767 µm, 13,345 ± 0,728 µm idi; KKBU ölçümleri 8,918 ± 0,471 µm, 9,414 ± 0,483 µm, 9,457 ± 0,476 µm olarak belirlendi. İnsan, koyun ve sığırlardan izole edilen büyük ve küçük kancaların uzunluk, genişlik ve bıçak uzunlukları tüm gruplar arasında önemli farklılık gösterdi. Kanca morfolojisi ölçümleri insan-koyun, insan-sığır ve koyun-sığır olarak gruplandırılıp istatistiksel olarak incelendiğinde; BKU, KKU, KKG ve KKBU'nun insan-sığır ve insan-koyun arasında anlamlı olarak farklı olduğu, ancak koyun-sığır arasında olmadığı belirlendi. LHW'nin insan-sığır ve koyun-sığır arasında önemli ölçüde farklı olduğu, BKBU'nun ise sadece insan-koyun arasında önemli ölçüde farklı olduğu bulundu.

Sonuç: *E. granulosus*'un büyük ve küçük kancalarının morfolojik özellikleri, omurgalı konakçılarda morfolojik adaptasyonu temsil edebilir. Bu nedenle, morfolojik parametrelerin izolatların ayrımında faydalı olabileceği ve ileride moleküler çalışmalar ile birlikte kullanılabileceği düşünülmektedir.
***Anahtar kelimeler*:** *Echinococcus granulosus*, Hidatik kist, Protoskoleks, Çengel, Morfoloji

**Corresponding author: Yunus Emre Beyhan. E-mail address:* yebeyhan@gmail.com.
ORCIDS: Ahmed Galip Halidi: 0000-0002-1780-6671, *Hasan Yılmaz:* 0000-0001-6947-4499, *Yunus Emre Beyhan:* 0000-0002-1696-4803
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INTRODUCTION

Cystic echinococcosis (CE) is an important parasitic infection caused by *Echinococcus granulosus* (*E. granulosus*) larvae, adults of which live in canidae. Although it is observed in all over the world, CE is more prevalent in developing and underdeveloped nations (Ahmadi and Dalimi, 2006; Latif et al., 2010). CE is especially an important disease in Mediterranean countries, and it is more prevalent in Turkey when compared to developed countries (Aydin et al., 2012; Aksu et al., 2013). CE is most often localized in liver and lungs and leads to clinical symptoms that vary based on the tissue or organ of localization. In addition to health problems that vary depending on the location of the CE, it causes high hospital costs and labor losses in people. In animal husbandry, it significantly damages national economy especially due to the waste of infected livers (Almeida et al., 2009; Singh et al., 2014; Mustafa et al., 2015).

Previously, it was considered that *E. granulosus* had 10 strains (G1-G10); however, molecular studies demonstrated that certain strains were in fact different species. The last classification was as follows: *E. granulosus* sensustricto (ss) (G1 - G3), *Echinococcus equinus* (G4), *Echinococcus ortleppi* (G5), *Echinococcus canadensis* genotypic cluster (G6, G7, G8 and G10; suspected G9) and *Echinococcus felidis* (lion strain) (Beyhan and Umur, 2011). Strain variations are effective on the life cycle, pathogenicity, host specificity, drug sensitivity, epidemiology of the disease, and the rate of parasite development. It is of great importance to determine the dominant strains in endemic regions for the control and eradication of the parasite (Eckert and Thompson, 1997). *E. granulosus* strains exhibit epidemiological, morphological, biochemical, physiological and genetic differences (Eslamiet al., 2014). The most important morphological difference is the variations in larval rostella hooks. Thus, *E. granulosus* larval strains could be identified by the examination of the morphological structure of the hooks lined up in the form of two rows on the protoscolex rostellum using the adequate method (Latif et al., 2009; Harandi et al., 2012; Mustafa et al., 2015).

The aim of the present study was to investigate and compare the hook morphology of protoscolex isolated from humans, sheep and cattle CE samples, and to determine the differences.

MATERIAL and METHOD

The present study was conducted on 60 liver cyst samples isolated from patients operated in University Medical Center with the diagnosis of CE (n=20), and waste organs isolated from slaughtered sheep (n=20) and cattle (n=20) between June 2018 and April 2019.

The cyst fluids were centrifuged at 1500 rpm for 5 minutes and protoscoleces were collected from the bottom. A wet mount preparation was obtained for each collected sample. For each of these hosts, 20 large and 20 small hooks were examined. Large hook length (LHL), small hook length (SHL), large hook width (LHW), small hook width (SHW), large hook blade length (LHBL), small hook blade length (SHBL) were measured. The measurements were made by a single person, taking into account the characteristics specified by Hobbs et al. (1990) as presented in Figure 1. Leica DM 750 research microscope was employed for the measurements.

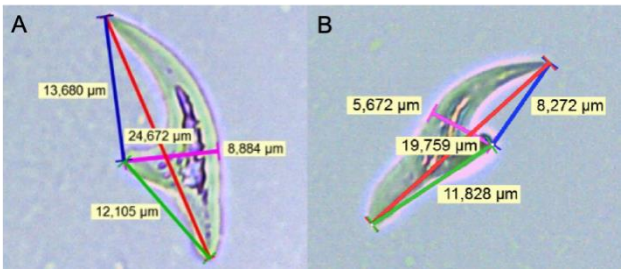

Figure 1. Measurement parameters for morphological analysis. (A: Large Hook, B: Small Hook; Red: Hook length, Pink: Hook width, Blue: Hook blade length)

Statistical Analysis

Shapiro-Wilk test was used to test of normality. According to results, non-parametric tests were preferred. Larval rostellar hook lengths were compared between 3 groups by using Kruskall Wallis test and multiple comparison tests (Bonferroni) were used to know which groups differ from which others. General descriptive statistics are summarized as mean±st. deviation for continuous variables. A "p" value of less than 0.05 was considered statistically significant and IBM SPSS Statistics for Windows, Version 20.0. were used for all these statistical analyses.

RESULTS

The mean human, sheep and cattle LHL measurements were 21.710 ± 1.073 µm, 24.322 ± 1.073 µm, 25.223 ± 1.073 µm, respectively. The mean SHL measurements were 16.946 ± 0.840 µm, 20.746 ± 0.911 µm, 21.199 ± 0.977 µm, respectively. The mean LHW measurements were 5.437 ± 0.358 µm, 7.817 ± 0.729 µm, 8.528 ± 0.589 µm, respectively. The mean SHW measurements were 7.229 ± 0.631 µm, 6.417 ± 0.507 µm, 6.488 ± 0.463 µm, respectively. The mean LHBL measurements were 13.236 ± 0.746 µm, 13.862 ± 0.767 µm, 13.345 ± 0.728 µm, respectively. The mean SHBL measurements were 8.918 ± 0.471 µm, 9.414 ± 0.483 µm, 9.457 ± 0.476 µm, respectively (Table 1).

The statistical comparison of the length, width and blade lengths of large and small hooks isolated from humans, sheep and cattle revealed significant differences ($P<0.05$) between all groups. The statistical analysis of paired hook morphology measurements such as human-sheep, human-cattle and sheep-cattle determined that LHL, SHL, SHW and SHBL measurements were significantly different

between human-cattle and human-sheep groups; however, no significant difference was determined between sheep and cattle. LHW was significantly different between human-cattle and sheep-cattle groups, and LHBL was significantly different ($P<0.05$) only between human-sheep (Table 1).

Table 1. Statistical analysis of mean differences of larval rostellar hook lengths in human, shep and cattle strains.

Measured	Host (n)	Mean ± SD	p*	Cross	p **
SHL (µm)	Human (20)	16,946 ± 0,840	0,001	Sheep Cattle	1,00
	Sheep (20)	20,746 ± 0,911		Human Cattle	0,001
	Cattle (20)	21,199 ± 0,977		Human Sheep	0,001
LHL (µm)	Human (20)	21,710 ± 1,073	0,001	Sheep Cattle	0,358
	Sheep (20)	24,322 ± 1,073		Human Cattle	0,001
	Cattle (20)	25,223 ± 1,073		Human Sheep	0,001
SHBL (µm)	Human (20)	8,918 ± 0,471	0,001	Sheep Cattle	1,00
	Sheep (20)	9,414 ± 0,483		Human Cattle	0,003
	Cattle (20)	9,457 ± 0,476		Human Sheep	0,009
LHBL (µm)	Human (20)	13,236 ± 0,746	0,036	Sheep Cattle	0,144
	Sheep (20)	13,862 ± 0,767		Human Cattle	1,00
	Cattle (20)	13,345 ± 0,728		Human Sheep	0,046
SHW (µm)	Human (20)	7,229 ± 0,631	0,001	Sheep Cattle	1,00
	Sheep (20)	6,417 ± 0,507		Human Cattle	0,001
	Cattle (20)	6,488 ± 0,463		Human Sheep	0,001
LHW (µm)	Human (20)	5,437 ± 0,358	0,001	Sheep Cattle	0,019
	Sheep (20)	7,817 ± 0,729		Human Cattle	0,001
	Cattle (20)	8,528 ± 0,589		Human Sheep	0,090

DISCUSSION

E. granulosus larvae induced CE is one of the important zoonotic diseases globally and in Turkey. CE leads to various clinical symptoms based on the tissue and organ of localization in humans, and it also affects the quality of life negatively and creates problems in national economy. Variations in *Echinococcus* species, life cycle of the parasite, host specificity, development rate, pathogenicity, antigenicity and sensitivity to chemotherapeutic agents play a key role in the transmission dynamics, disease epidemiology and control methods (McManus and Thompson, 2003; Beyhan and Umur, 2011). DNA-based molecular techniques are sensitive and reliable tools for the determination of the nature of the variations among *Echinococcus* species. Furthermore, molecular and morphological variations are believed to be complementary in epidemiological studies on CE. Protoscolex hook morphometry could be used as an alternative and complementary for the identification of *E. granulosus* species in studies where large number of isolates are surveyed in endemic areas due to the high cost and limited availability of molecular methods. Although different rostella hook characters have been investigated in previous studies, it was determined that the small and large hook lengths were the most adequate characteristics for strain identification (Harandiet al., 2012).

In recent years, several studies were conducted on the molecular differences between the parasite strains and nucleotide differences were determined; however, there were limited studies on morphological variations. It was observed that it was possible to determine the parasite strains with the morphological analysis of *E. granulosus* protoscoleces (Ahmadi, 2004; Yildiz and Gurcan, 2009; Elmajdoub

et al., 2014; Singh et al., 2014) The protoscolex and hook morphology was investigated in sheep and camel CE isolates and morphology was determined to be significant in strain identification. It was suggested that the differences between certain samples were due to mutations in that region and morphometric properties could be used along with molecular studies (Elmajdoubet al., 2014).

The morphometric properties of protoscolex hooks isolated from cattle (Almeida et al., 2009) and human liver cyst samples (Almeida et al., 2015) were analyzed. In both studies, various mutations were identified in the large and small hooks of protoscolex. In the present study, it was determined that the hook morphologies in human, sheep and cattle isolates exhibited statistically significant differences. This finding was consistent with previous study results (Ahmadi, 2004; Almeida et al., 2009; Yıldız and Gurcan, 2009; Elmajdoub et al., 2014; Singh et al., 2014; Almeida et al., 2015) . Hussainet al. (2005) were reported that the parameters obtained in the morphometric analysis of protoscolex isolated from cattle and sheep were statistically similar. Yıldız and Gurcan (2009) were the first and an only study that compared the morphology of the sheep and cattle isolate larval hooks in Turkey. They found that the morphometric characteristics were quite similar in sheep and cattle isolates, except LHBL parameter was statistically different. In parallel with their study, we concluded that sheep and cattle may be infected with the same *E. granulosus* strain in Turkey.

In certain studies, conducted on protoscoleces from humans (Weatman and Williams, 1963; Hobbs et al., 1990; Gordo and Bandera, 1997;), were reported that the hook structures were shorter than the animal samples. Hama and Shareef (2016) were investigated the larval hook structures of human, sheep and cattle isolates and they reported the length and width of the hook blade of the isolates were significantly different. This indicate that, different results can be obtained depending on the geographical region where the study is conducted.

Harandiet al. (2012), conducted morphological and genetic comparisons on the protoscoleces isolated from animals and human samples. They were reported that only the large hook length could be used in the separation of G3 and G6 genotypes; however, the hook morphology could not be used to distinguish G1 and G3 strains. The morphological differences were identified between human and animal. LHL, SHL, SHW and SHBL were different between human-cattle and human-sheep isolates, LHW was different between human-cattle and LHBL was different between human-sheep isolates ($P < 0.05$).

Conclusions

The present study was the first study conducted on the hook morphology of human *E. granulosus* isolates in Turkey. In conclusion, the morphological features of the small and large hooks of *E. granulosus* may represent morphological adaptation in vertebrate hosts. Thus, morphological parameters could be useful in future studies. It is thought that such morphological studies should be disseminated especially in regions where CE is endemic, and morphological results in addition to molecular methods will be important for obtaining information about the strains and for the epidemiology of the disease.

Conflict of interest

There is no conflict of interest.

Financial Disclosure

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Ethical Approval

The present study with the recordings of the participants has been approved by Experimental Animal Ethical Committee belonging to Van YYU (approval number: 2022/01-09).

REFERENCES

Ahmadi N, Dalimi AJI (2006). Characterization of *Echinococcus granulosus* isolates from human, sheep and camel in Iran. *Genetics and Evolution,* 6, 85-90.

Ahmadi N (2004). Using morphometry of the larval rostellar hooks to distinguish Iranian strains of *Echinococcus granulosus*. *Parasitology,* 98, 211-220.

Aksu M, Kırcalı Sevimli F, İbiloğlu İ, Bozdoğan Arpacı R (2013) Mersin ili'nde kistik ekinokokkozis (119 olgu). *Türkiye Parazitoloji Dergisi*, 37, 252-256.

Almeida F, Oliveira F, Neves R, Siqueira N, Rodrigues-Silva R, Daipert-Garcia D et al. (2015). Morphometric characteristics of the metacestode *Echinococcus vogeli* Rausch & Bernstein, 1972 in human infections from the northern region of Brazil. *Journal of Helminthology*, 89, 480-486.

Almeida F, Silva RR, Neves R, Gonçalves M, Romani E, da Silva JRMC (2009). Morphological and morphometric studies on protoscoleces rostellar hooks of Echinococcus granulosus from Peru visualized by several microscopic techniques. *Neotropical Helminthology*, 3, 65-71.

Aydın M, Adıyaman G, Doğruman-Al F, Kuştimur S, Ozkan S (2012). Determination of anti-echinococcus IgG antibodies by ELISA in patients with suspected hydatid cyst. *Türkiye Parazitoloji Dergisi*, 36(2), 61-64.

Beyhan YE, Umur Ş (2011). Molecular characterization and prevalence of cystic echinococcosis in slaughtered water buffaloes in Turkey. *Veterinary Parasitology*, 181, 174-179.

Elmajdoub LO, Rahman WA, Fadzil M, Mohd SA (2014). Studies on the protoscoleces and hooks of Echinococcus granulosus from Libya by scanning electron microscope. *Acta Medica International*, 1, 74-81.

Eslami A, Shayan P, Bokaei S (2014). Morphological and genetic characteristics of the liver hydatid cyst of a donkey with Iran origin. *Iran Journal of Parasitology,* 9, 302-310.

Gordo FP, Bandera CC (1997). Differentiation of Spanish strains of *Echinococcus granulosus* using larval rostellar hook morphometry. *International Journal for Parasitology*, 27, 41-49.

Harandi MF, Hajialilo E, Shokouhi M (2012). Larval hook length measurement for differentiating G1 and G6 genotypes of Echinococcus granulosus sensu lato. *Türkiye Parazitoloji Dergisi*, 36, 215-218.

Hobbs R, Lymbery A, Thompson RC (1990). Rostellar hook morphology of *Echinococcus granulosus* (Batsch, 1786) from natural and experimental Australian hosts, and its implications for strain recognition. *Parasitology,* 101, 273-281.

Hussain A, Maqbool A, Tanveer A, Anees A (2005). Studies on morphology of *Echinococcus granulosus* from different animal-dog origin. *Punjab University Journal of Zoology*, 20, 151-157.

IBM Corp. Released 2011. IBM SPSS Statistics for Windows, Version 20.0. Armonk, NY: IBM Corp.

Latif A, Tanveer A, Riaz-Ud-Din S, Maqbool A, Qureshi AW (2009). Morphometry of protoscoleces rostellar hooks of *Echinococcus granulosus* isolates from Punjab, Pakistan. *Pakistan Journal of Science,* 61, 223-228.

Latif AA, Tanveer A, Maqbool A, Siddiqi N, Kyaw-Tanner M, Traub R (2010). Morphological and molecular characterisation of *Echinococcus granulosus* in livestock and humans in Punjab, Pakistan. *Veterinary Parasitology,* 170, 44-49.

McManus D, Thompson RC (2003). Molecular epidemiology of cystic echinococcosis. *Parasitology*, 127, 37-51.

Mustafa I, Shahbaz M, Asif S, Khan MR, Saeed U, Sadiq F, Mehmood T, Ahmed H, Simsek S (2015). Availability, cyst characteristics and hook morphology of *Echinococcus granulosus* isolates from livestock (cattle, sheep and goats) in Central Punjab, Pakistan. *Kafkas Universitesi Veteriner Fakultesi Dergisi,* 21, 849-854.

Singh BB, Sharma JK, Tuli A, Sharma R, Bal MS, Aulakh RS et al. (2014). Prevalence and morphological characterisation of Echinococcus granulosus from north India. *Journal of Parasitic Diseases*, 38(1), 36-40.

Sweatman G, Williams R (1963). Comparative studies on the biology and morphology of *Echinococcus granulosus* from domestic livestock, moose and reindeer. *Parasitology*, 53(3-4), 339-390.

Yıldız K, Gurcan IS (2009). The detection of *Echinococcus granulosus* strains using larval rostellar hook morphometry. *Türkiye Parazitoloji Dergisi*, 33(3), 199-202.